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Heritability of Testis Size

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Testis size is an important feature of male pubertal development. The genetic and environmental contributions to variation in human testis size have hardly been studied. We estimated the heritability of human testicular size in a group of mono- and dizygotic twins and their non-twin brothers (145 twins and 20 brothers from 95 families). Participants were 18 years old on average and all had reached Tanner development stage 4 or higher. Dizygotic twins and their siblings had a larger mean testis volume than monozygotic twins and their siblings. There was significant familial resemblance, with higher correlations in monozygotic twin pairs (0.59) than in dizygotic twin and sibling pairs (0.34). Heritability was estimated at 59% (95% CI = 37–75%), but a model that excluded genetic influences and attributed all familial resemblance to shared environment, fitted the data only marginally worse. The finding of larger mean testis volume in dizygotic twins may be of interest for future research into the mechanisms underlying dizygotic twinning.

Keywords: familial effects, genetics, testicular volume, twins

Testis size is one of the hallmarks of male pubertal development. The beginning of testicular enlargement is usually the first sign of puberty in boys. Therefore, the measurement of testis size is of great clinical importance when analyzing growth (disorders) in boys. Several studies have investigated testicular volume, which has resulted in the availability of nationwide reference values (Mul et al., 2001).

Testis size is related to testicular function. The number of Sertoli cells present determines both testis size and sperm output (Petersen & Soder, 2006) and thus is of importance for male fertility. However, little is known about the causes of individual differences in human testis volume. Ethnic differences in human testis size have been described (Short, 1984), testes being larger in Caucasian than in Asian men, which suggests that genetic influences play a role in the variation of human testis size. The pre- and perinatal period is known to be important for testicular development (Orth et al., 1988), implying a contribution of environmental factors to the variation in testis size as well. To gain more insight into the determinants of testis size, we will explore the genetic and environmental influences on the variation in testis size. One small pilot study in Australian twins investigated the genetic contribution to variability in human testicular function and size (Handelsman, 1997). A strong familial effect was seen, but a genetic component could not be confirmed due to the small number of participants (11 monozygotic (MZ) and 6 dizygotic (DZ) twin pairs). The aim of the current study is to estimate the genetic and environmental contribution to the variation in testis size in a sample of 18-year-old twins and their adolescent siblings.

Materials and Methods

Subjects

All participants were contacted via the Netherlands Twin Register (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam, the Netherlands (Boomsma et al., 1992; Boomsma et al., 2006). The current study sample is part of a longitudinal project on physical and mental development and comprises 184 families of 18-year-old twin pairs and their siblings (Bartels et al., 2002). The initial sample was composed of 164 male twins and 46 of their male siblings. Data on testis size were available for 148 twins and 24 male siblings from 97 families. We excluded data for several reasons: orchidectomy (1 twin); traumatic injury (1 twin); torsio testis (1 twin). For analyses, we selected subjects in Tanner genital development stage 4 and above (145 twins and 20 siblings from 95 families). Mean age at assessment was 18.17 years (*SD* 0.21) in the twin group and 17.68 years (*SD* 3.09) in the sibling group. One twin pair had Surinam–Hindustan parents and 2 twin pairs had an Indonesian parent. Although ethnic variation in testis size has been described, we did not exclude these subjects as their testis size was within normal range compared to Dutch reference values

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Table 1

Mean Total Testis Size (ml) of Twins and Siblings From MZ (Monozygotic) and DZ (Dizygotic) Families

	N	Total testis size mean	*SD*
MZ twins + siblings	62	15.32	6.68
DZ twins + siblings	103	17.96	4.88

Note: *SD* is standard deviation.

(Mul et al., 2001).The final sample comprised 30 MZM (monozygotic male) and 32 DZM (dizygotic male) twin pairs, 33 DOSM (dizygotic opposite sex male) twins and 20 siblings (7 MZ siblings and 13 DZ siblings). The zygosity of the same-sex twin pairs (62 pairs) was determined by DNA analyses (59 pairs) or blood group polymorphisms (3 pairs).

This study was approved by the Central Committee on Research Involving Human Subjects. Written informed consent was obtained from all participants and also from all parents of underage participants.

Pubertal Development

All participants filled out the Dutch Health and Behaviour Questionnaire (Bartels et al., 2007a), a large self-report questionnaire including the pubertal development scale (Peterson et al., 1988). In addition, stage of puberty of all subjects was physically determined by the same trained researcher on the basis of secondary sexual characteristics using the stages of development devised by Tanner (Marshall & Tanner, 1970). Left and right testicular volume was measured using a Prader orchidometer, in which a series of testes models of volumes 2, 3, 4, 6, 8, 10, 12, 15, 20 and 25 ml were compared tactually with the actual testis (Zachmann et al., 1974). Total testis volume was calculated by taking the sum of left and right testis volume.

Data Analysis

All analyses were carried out using structural equation modelling in the software package Mx (Neale et al., 2006). Saturated models in Mx were used to test for the effect of zygosity on mean and variance of total testis size and for differences between twins and siblings in mean and variance of total testis size. Twin and twin-sibling correlations for the two zygosity groups (MZ-DZ) were estimated. The twin–sibling correlation was constrained to equal the DZ correlation. All analyses were conducted using raw data. Age at measurement was included as a covariate.

Genetic Modelling

Mx was also used to carry out genetic analyses. The variation in total testis size was decomposed into sources of additive genetic variance (A), common environmental variance (C) and unique environmental variance (E). A is due to additive genetic effects of different alleles, C is due to common environmental influences shared by individuals from the same family, and E is due to unique (nonshared) environmental influences. E also includes measurement error and is always included in the models. Based on the twin and twin-sibling correlations an ACE model was fitted to the data. Significance of the A and C components were tested by dropping the component from the model. Submodels were compared to the full ACE model using the likelihood ratio test.

Results

Table 1 provides mean testicular volume of MZ and DZ twins and their siblings derived from the saturated model. Information on pubertal stage is shown in table 2. Forty-one twins (28%) and 11 siblings (55%) reported that their testes were still growing. One twin reported not knowing whether his testes were still growing. The majority of twins and siblings were in the final pubertal stage (pubic hair stage 6 or genital development stage 5). All twin pairs were concordant for genital development stage.

Age (years) affected testis volume (ml) significantly ($\chi^2 = 16.41$, $df = 1$, $p < .01$; b = 1.54). There was a significant effect of zygosity on the mean and variance. Average testis volume and variance were larger in DZ than in MZ twins (mean: $\chi^2 = 4.10$, $df = 1$, $p = .04$; variance: $\chi^2 = 4.89$, $df = 1$, $p = .03$). There were no differences between twins and siblings within zygosity groups in mean ($\chi^2 = 1.14$, $df = 2$, $p = .57$) or variance of testis size (χ^2=0.52, $df = 2$, $p = .77$). The twin correlation for testis volume was $r = .59$ (CI .34–.75) in MZ pairs and $r = .34$ (CI –.05–.59) in DZ twin and sibling pairs. The higher MZ twin correlations than

Table 2

Pubertal Development of Twins and Siblings; DHBQ is Dutch Health and Behaviour Questionnaire (Bartels et al., 2007a)

	DHBQ			Tanner stages				
	Testes still growing?			Genital development		Pubic hair development		
	Yes	No	Do not know	4	5	4	5	6
	N	*N*	*N*	*N*	*N*	*N*	*N*	*N*
Twins	41	103	1	1	140	5	23	115
Siblings	11	9	0	6	13	7	2	10

Table 3

Genetic Model Fitting Results for Total Testis Size

	–2LL	*df*	χ^2	Δdf	c.t.m.	*p*	AIC
Total testis size							
1. Full model ACE	1016.30	159					
2. AE (no C)	1016.36	160	0.06	1	1	0.81	–1.94
3. CE (no A)	1018.38	160	2.08	1	1	0.15	0.08
4. E	1036.54	161	20.24	2	1	<0.01	16.24

Note: –2LL = –2 log likelihood, df = degrees of freedom, χ^2 = chi-square statistic, Δdf = difference in degrees of freedom, c.t.m. = compared to model, *p* = probability-value, AIC = Akaike's Information Criterion, A = additive genetic influences, C = common environment, E = unique environment

DZ twin/sibling correlations indicate an influence of genetic factors on testis volume. However, since the MZ correlation is less than twice the DZ correlation influences of shared environment are to be expected.

Table 3 gives the results for the genetic modelling. Both the AE (p = .81, AIC = –1.94) and CE model (p = .15, AIC = 0.08) (with age as a covariate) fitted the data well. Dropping both the additive genetic and common environmental factors to zero caused a significant worsening of fit (p < .01), indicating significant familial resemblance for testis size.

Variance component estimates are provided in Table 4, as well as the standardized estimates with the 95% confidence intervals. In the full ACE model 50% of the variation in total testis size is explained by genetic factors, while common environmental influences accounted for 9% of the individual differences. The confidence intervals around the standardized estimates for V_A and V_C show an overlap including zero, indicating a lack of statistical power to differentiate between genetic and common environmental influences.

Discussion

This study examined the genetic and environmental influences on testis size in late adolescence. Our data showed significant familial resemblance, with higher correlations in MZ twin pairs than in DZ twin and sibling pairs. Heritability of testis size was estimated at 59%. We were not able to differentiate between genetic and shared environmental effects due to a lack of statistical power. A model that excluded genetic influences and attributed all familial resemblance to shared environment fitted the data only marginally worse than a genetic model. Evidence for genetic influences on testis size comes from a study of inbred mouse strains (Chubb, 1992). It showed that there are genes that control testis size by regulating the number of Sertoli cells.

A remarkable finding is the larger testis volume in DZ twins. DZ twins had a significantly larger testis volume than MZ twins, both zygosity groups having a mean testis volume within the normal range (between the 50th and 90th percentile of Dutch reference values) (Mul et al., 2001). It has been hypothesized that a lower incidence of DZ twinning, within an ethnic population, may be correlated with lower average testis size in that population (Short, 1984). Asian populations have lower DZ twinning frequencies and smaller testis size compared to Caucasian populations. Another indication for a possible relation between DZ twinning and testis size comes from a Belgian study (Fryns, 1986), which reported an increase in DZ twinning in the offspring of female carriers of the Fragile X. Large testes are one of the characteristic features of Fragile X mental retardation syndrome. These observations lead to the hypothesis that there may be genetic factors responsible for both DZ twinning and larger testis size. The follicle-stimulating hormone (FSH) may play a role in the association: mothers of DZ twins have shown increased FSH concentrations (Lambalk et al., 1998; Martin et al., 1984; Nylander, 1974), and smaller testes have been observed in men who lack a functional FSH receptor (Tapanainen et al., 1997). However, results from a study using a mouse model for the Fragile X syndrome (Slegtenhorst-Eegdeman et al., 1998), showed that macro-orchidism is caused by an increased rate of Sertoli cell proliferation in the

Table 4

Standardized and Unstandardized Estimates of Variance Components for Total Testis Size With 95% Confidence Intervals Between Brackets

	Standardized			Unstandardized		
Total testis size	V_A	V_C	V_E	V_A	V_C	V_E
ACE	.50 (.00–.75)	.09 (.00–.59)	.41 (.25–.66)	15.43	2.60	12.58
AE	.59 (.37–.75)	—	.41 (.25–.63)	18.07	—	12.35
CE	—	.48 (.27–.64)	.52 (.36–.73)	—	15.00	16.46

Note: V_A = variance explained by additive genetic factors (heritability), V_C = variance explained by common environment, V_E = variance explained by unique environment

embryonic and early postnal period, which appeared not to be the result of a major change in FSH signal transduction. The larger testis size in DZ twins may be of interest in unravelling the mechanisms underlying DZ twinning.

Another explanation for the difference in mean testis volume between MZ and DZ twins could be that MZ twins generally experience more pre- and perinatal problems than DZ twins (Dube et al., 2002). The pre- and perinatal period is of great importance for testicular development, as Sertoli cells divide rapidly and extensively during fetal and early postnatal life (Orth et al., 1988). After adjusting for birth weight and gestational age we still found DZ testes to be significantly larger than MZ testes (data not shown).

A study of testis size in primates showed testis weight to increase with body weight (Harcourt et al., 1981). In our data, no significant correlation was demonstrated between body weight and testis size nor did the MZ-DZ differences in mean testis size disappear after adjusting for body weight (data not shown).

Since twins may not be representative of singletons, as they experience more often pre- and perinatal problems due to intra-uterine growth retardation and prematurity, it is very important to include siblings as well. In our study no difference was shown in testis size between twins and siblings, although their number was small.

Testis size was measured using a Prader orchidometer, which has been reported to be less accurate than ultrasound measurements (Sakamoto et al., 2007a; Sakamoto et al., 2007b). They showed that the orchidometer overestimated the testicular volume, especially in small testes. However, testicular volume estimated by Prader orchidometry correlated closely with the measurements by ultrasonography (I = 0.7 to 0.8).

Furthermore, all subjects in our study have been examined by the same trained researcher, excluding the possibility of inter-observer variability. However, this may led to correlated measurement errors which are reflected in the C component in the genetic modelling (Bartels et al., 2007b). Such a form of rater bias could explain part of the estimated common environmental influences on testis size. Unfortunately, we did not have the information to investigate the influence of rater bias on our results.

In summary, this study provides evidence that the variation in testis size is influenced by familial effects. Unfortunately, we were not able to differentiate between genetic and common environmental influences due to a lack of power. More knowledge of the genetic and environmental effects on the variation in testis size may help us in understanding testicular function and fertility problems. The finding of larger mean testis volume in DZ twins may be of interest for future research into the mechanisms underlying DZ twinning.

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